ENGINEERING EXPERIMENT STATION
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FINAL REPORT

PROJECT NO. B-108 AND B-113

A STUDY OF THE TOXICITY OF CHROMIUM IN SEWAGE TREATMENT PROCESSES

By

ROBERT S. INGOLS AND ROBERT H. FETNER

GRANT-IN-AID RG 4363
FROM NATIONAL INSTITUTES OF HEALTH

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This report contains 26 pages.

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I. SUMMARY

The work on these research grants from the Surgeon General of the Public Health Service through the National Institutes of Health has studied the toxicity of chromium in the sewage treatment processes of activated sludge and of sludge digestion. With activated sludge, the toxicity of chromic and chromate ions were studied under conditions with various food to sludge concentration ratios with three different temperatures and with various types of food. The results were confusing at first until the full significance of the period of observation was understood. The anaerobic studies with sludge digestion indicated the importance of the ratio of chromate to organic matter and the general biological activity of the sludge. Because these sewage treatment materials do not permit an accurate evaluation of the number of organisms, pure cultures were studied under aerobic conditions. An interpretation of all of the results from aerobic conditions indicates that chromate at low concentrations is most toxic under those conditions which most favor reproduction and that chromate ions are much more toxic than chromic ions under these conditions. Under those conditions of slow growth where chromate ions show very little toxicity, chromic ions may be as toxic or more toxic than chromate ions. Under anaerobic conditions, the presence of chromate ions almost completely inhibits sludge digestion, while the time required for reducing smaller amounts of the chromate ion determines the extent of gas production.

II. INTRODUCTION

The research work which has investigated the toxicity of chromium in sewage treatment processes has been supported by a grant-in-aid from the Surgeon

General of the U. S. Public Health Service through the National Institutes of Health.

The primary object was to gain an understanding of the effect of chromium as both chromate and chromic ions on sewage digestion and the activated sludge process. A large number of digested sludges were tested with various amounts of sodium or potassium chromate; the rate of digestor gas production was used as a measure of response. The activated sludges were developed artificially in the laboratory; the rate of respiration (production of carbon dioxide) was used to evaluate the response of the organisms. Various types of sludges were produced by varying the type of feed used and the temperature of incubation. Finally, respiration responses of pure cultures of two bacteria, a yeast and a protozoan were compared with the number of organisms surviving.

III. PERSONNEL

The following personnel worked on the project:

R. S. Ingols, Project Director, Ph. D., Research Professor

R. H. Fetner, Associate Project Director, Ph. D., Assistant Research Biologist

L. T. Hilley, Chemist, A. B. (resigned 31 August 1956)

F. L. Britt, Chemist, A. B. (with project at its completion)

J. C. Meredith, Graduate Student Assistant, B. Sc (results were used for master's thesis)

D. Lillie, B. Warren, C. Donaldson, and J. D. Lupton: Student Assistants

IV. LABORATORY FACILITIES

In general, very little modification of existing laboratory facilities has been required by the work on this research. A four-point potentiometric recorder was changed to an eight-point recorder in order to obtain better comparison of toxicity factors under anaerobic conditions.

V. EXPERIMENTAL RESULTS

The study has been divided into four parts: the effect of chromium salts upon sewage sludge digestion, the effect of chromium salts upon the respiration of activated sludge, the use of pure cultures under aerobic conditions, and the effect of chromium upon the ability of activated sludge organisms to change from aerobic to anaerobic conditions.

A. Anaerobic Studies

The effect of the chromium salts upon sludge digestion was studied in seven different runs in which the normal laboratory procedure for batch sludge digestion studies was used. All digesting mixtures were prepared by mixing two parts by weight of matter from digested sludge with one part by weight of organic matter from fresh sludge. This gave a digestion which was nearly complete in 2 weeks. Extremely large variations were noted in the toxic biological responses.

The sludge digestion experiments were somewhat difficult to set up because of the unexpected variability in the response of the sludges to the same concentration of chromium. Thus, the data of the first run (as shown in Figure 1) indicated that there was very little depression in gas production from one gram of chromium (as sodium chromate) per liter of sludge mixture; in the next run, however, 200 mg chromium per liter had a marked depression on gas production

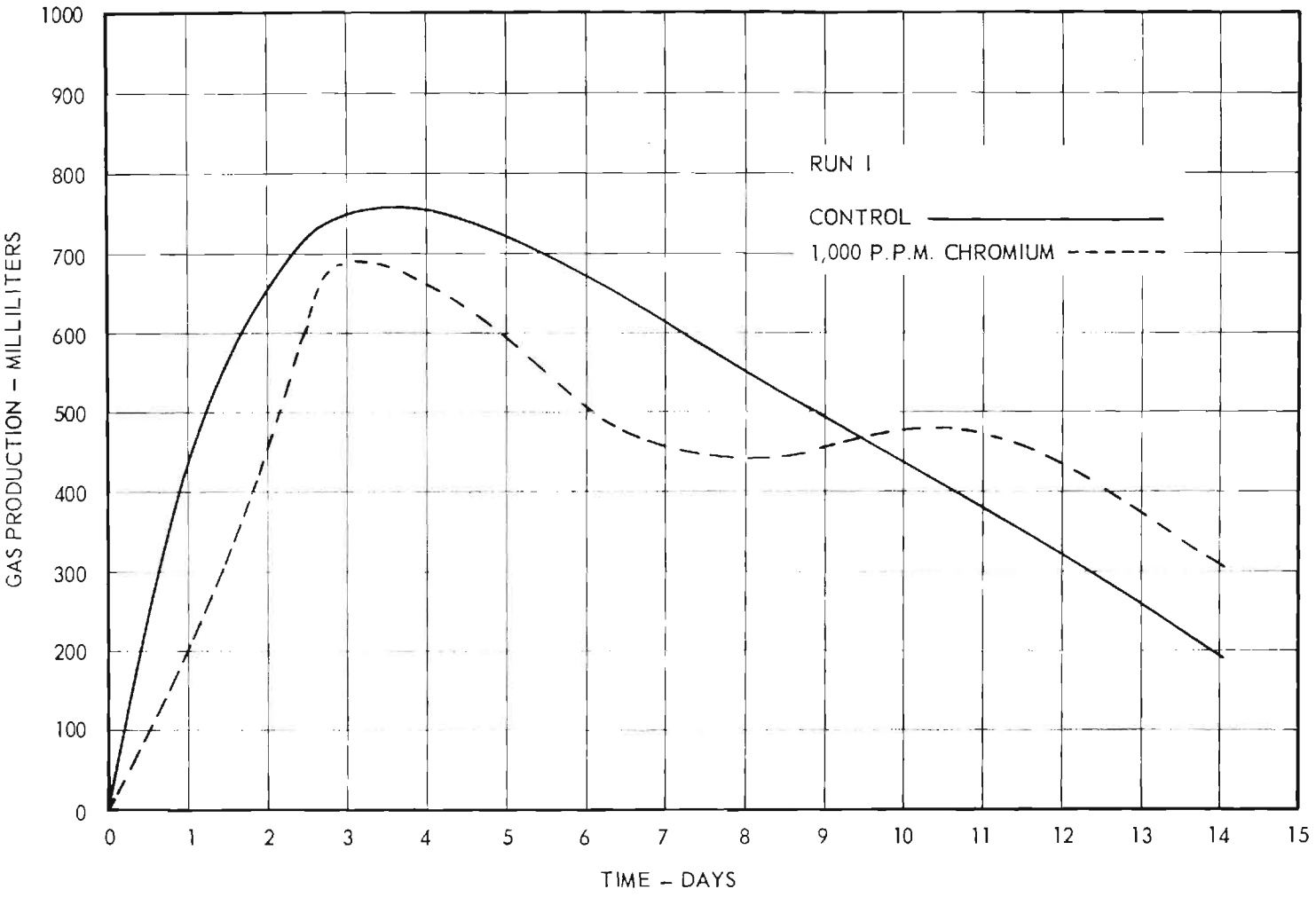

Figure 1. Effect of 1,000 ppm Chromium on Gas Production in Run I.

while 400 mg chromium per liter rendered the mixture almost inactive, as shown by the gas. These variations caused some difficulty in planning future experiments so that variations in the environment could be produced that would yield an effect which was adequate yet not excessive for measurement. A third experiment, at several concentrations and at different temperatures (as shown in Figure 3), indicated that 400 mg chromium per liter would give a sufficient change at 22° C and 30° C while at 37° C there was much less effect. In each of the previous experiments the chromium was added to the sludge mixture before the digestion was started.

Because the chromium was much less toxic when the control digestion mixture became most active immediately, it seemed that the time of adding the chromium should be important. When 400 mg per liter chromium (as chromate) was added immediately to a sludge digesting mixture the toxicity was great enough that the mixture did not recover appreciably in 19 days and very little gas was produced, as shown in Figure 4. When the same amount of chromium was added during the peak activity of the mixture there was a strong toxic effect followed by some recovery with daily gas production evident at 19 days when the experiment was terminated. When the same amount of chromium was added after the peak of gas production, the effect was much more severe, with recovery so much less that virtually no gas was being produced daily at the end of the run.

In the next run it was found that a sludge digesting mixture can tolerate much larger quantities of chromium that are added in small daily amounts rather than in one initial dose. Thus, 400 mg chromium per liter is much more toxic when added initially than 700 mg chromium per liter when added in seven daily increments of 100 mg per liter (see Figure 5).

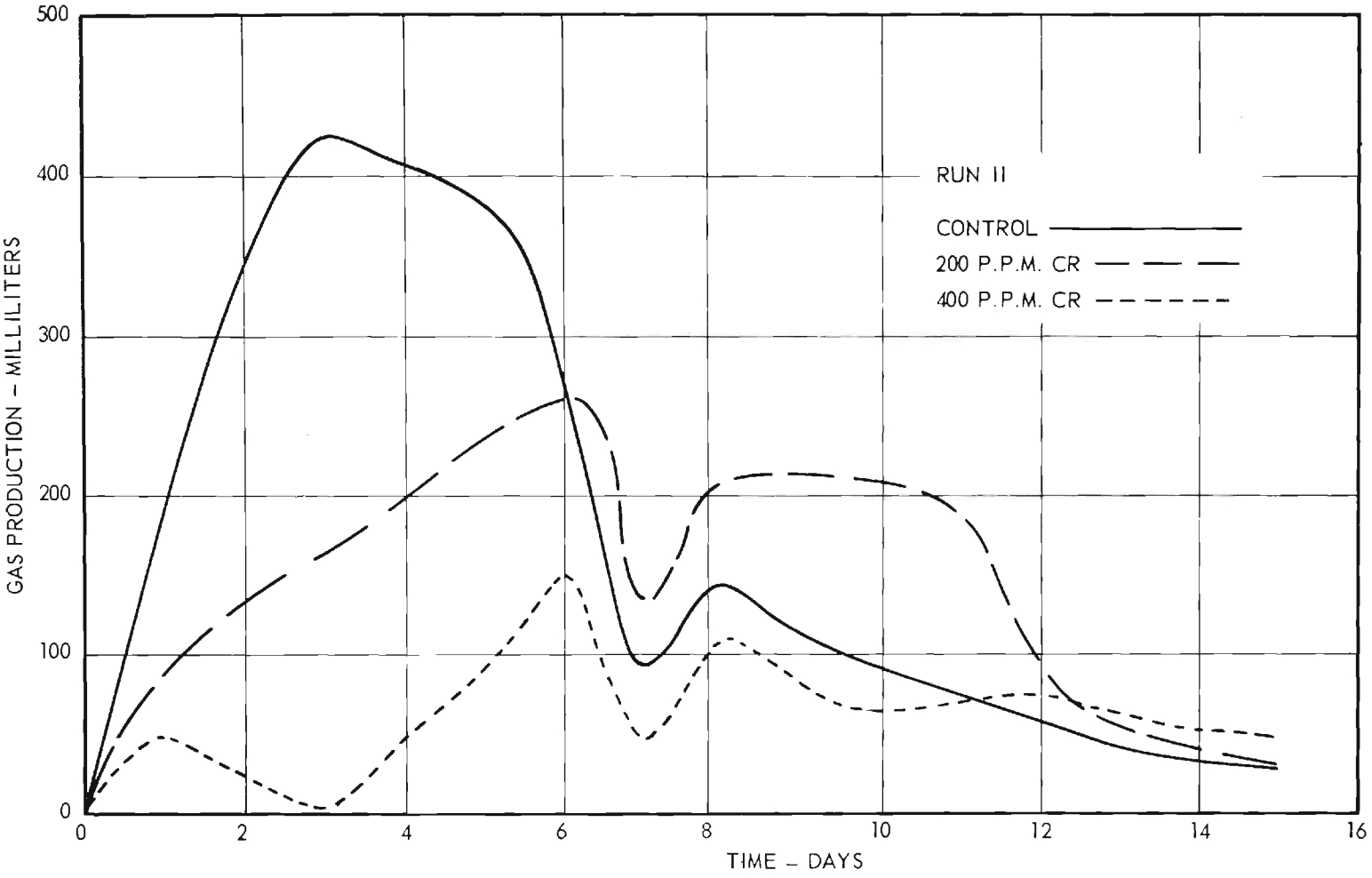

Figure 2. Effect of 200 ppm and 400 ppm Chromium on Gas Production in Run II.

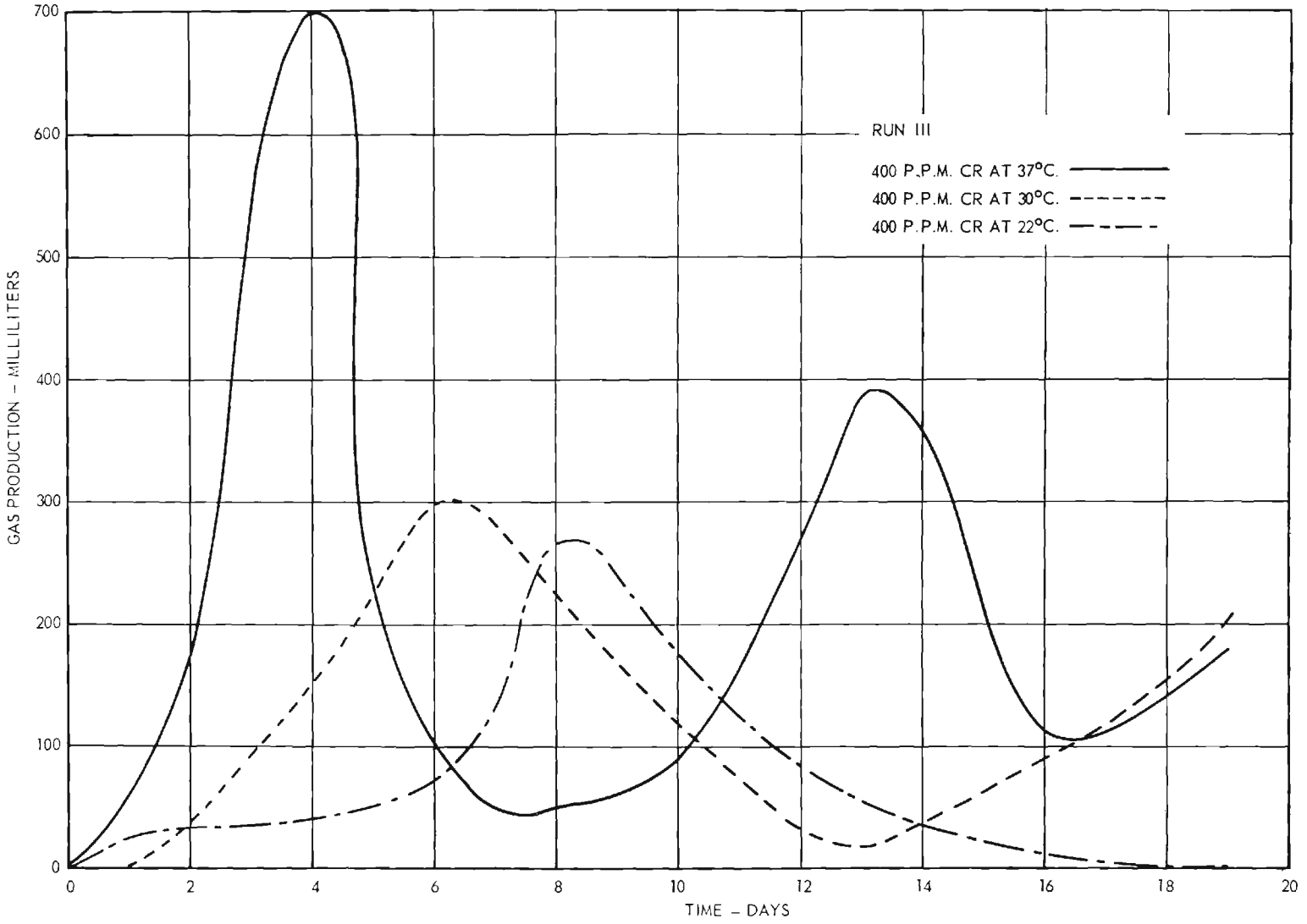

Figure 3. Comparison of Gas Production by Samples Containing 400 ppm Chromium at Three Different Temperatures in Run III.

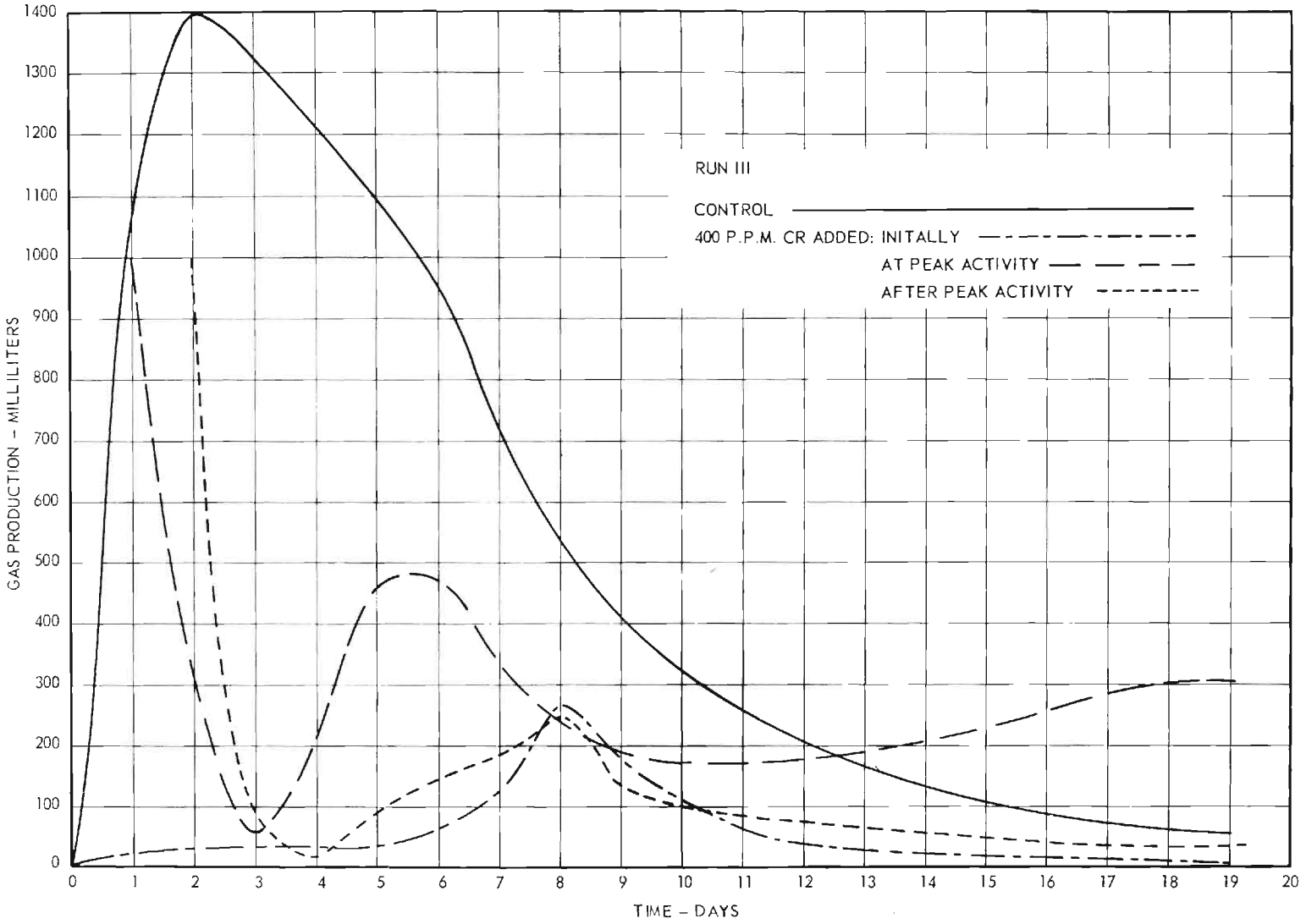

Figure 4. Comparison of Effects on Gas Production from Adding 400 ppm Chromium at Three Different Times During Digestion in Run III.

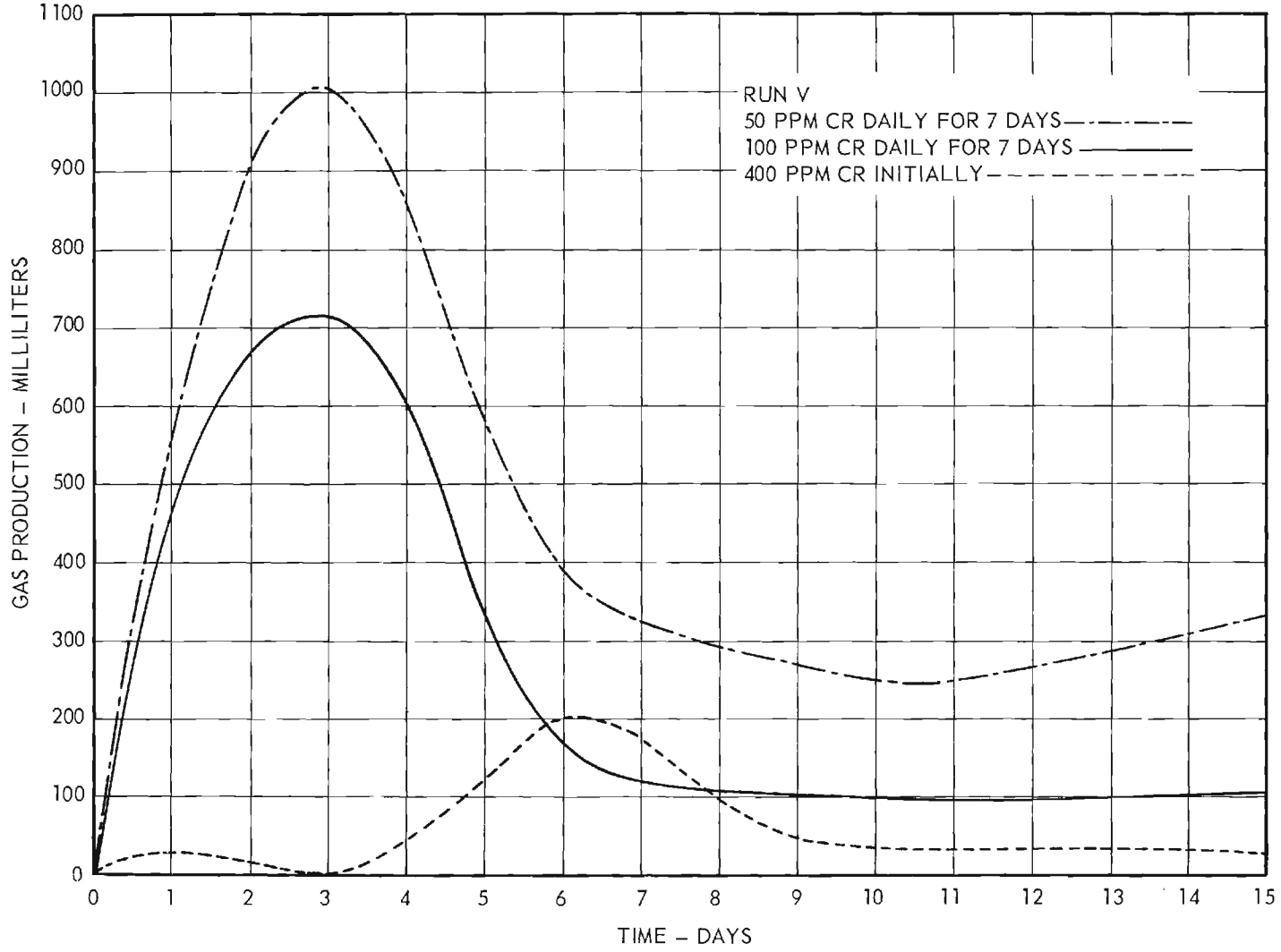

Figure 5. Comparison of Gas Productions of Samples with 400 ppm Chromium Added Initially, 50 ppm Chromium Added Daily, and 100 ppm Chromium Added Daily in Run V.

Through all of these runs it was observed that the reduction of the chromate was essential to the recovery of the digestion process. This supports earlier observations by the senior investigator and other research reported in the literature that the persistent yellow color of the chromate ion is correlated with a lack of gas. This would indicate that the chromic ion should be much less toxic than the chromate ion and this is undoubtedly true, but it is not simple to demonstrate this point experimentally. When chromic ions are added to a sludge digesting mixture, anions must be added in an equivalent amount. Sulfates produce sulfides which have been shown to be toxic; nitrates are reduced to nitrogen but interfere in methane production because of the use of the oxygen to oxidize the organic matter rather than permit its reduction to methane (there is also a possible redox potential toxicity to methane production by nitrates). Chromic chloride is dissolved with difficulty, but even if dissolved and added, it will leave a strong, stable, highly ionized acid in solution to affect the pH of the sludge mixture. Despite these hampering factors, evidence was obtained that the chromate is much more toxic than the chromic ion at 200 mg per liter chromium (see Figure 6). Because sludge concentrations varied over the period of study and because all additions of chromium were made on a volume basis rather than a weight of sludge basis, a study was made of the relationship of a constant, initial concentration of chromate to the various amounts of sludge present. The results of this study are shown in Table I.

The values of this table show that the ratio of chromate to the organic content of the sludge is more important than the concentration of chromate in the medium. The toxicity of the chromate, as indicated by the percentage reduction in gas produced, exceeds by a very large factor the reduction in gas

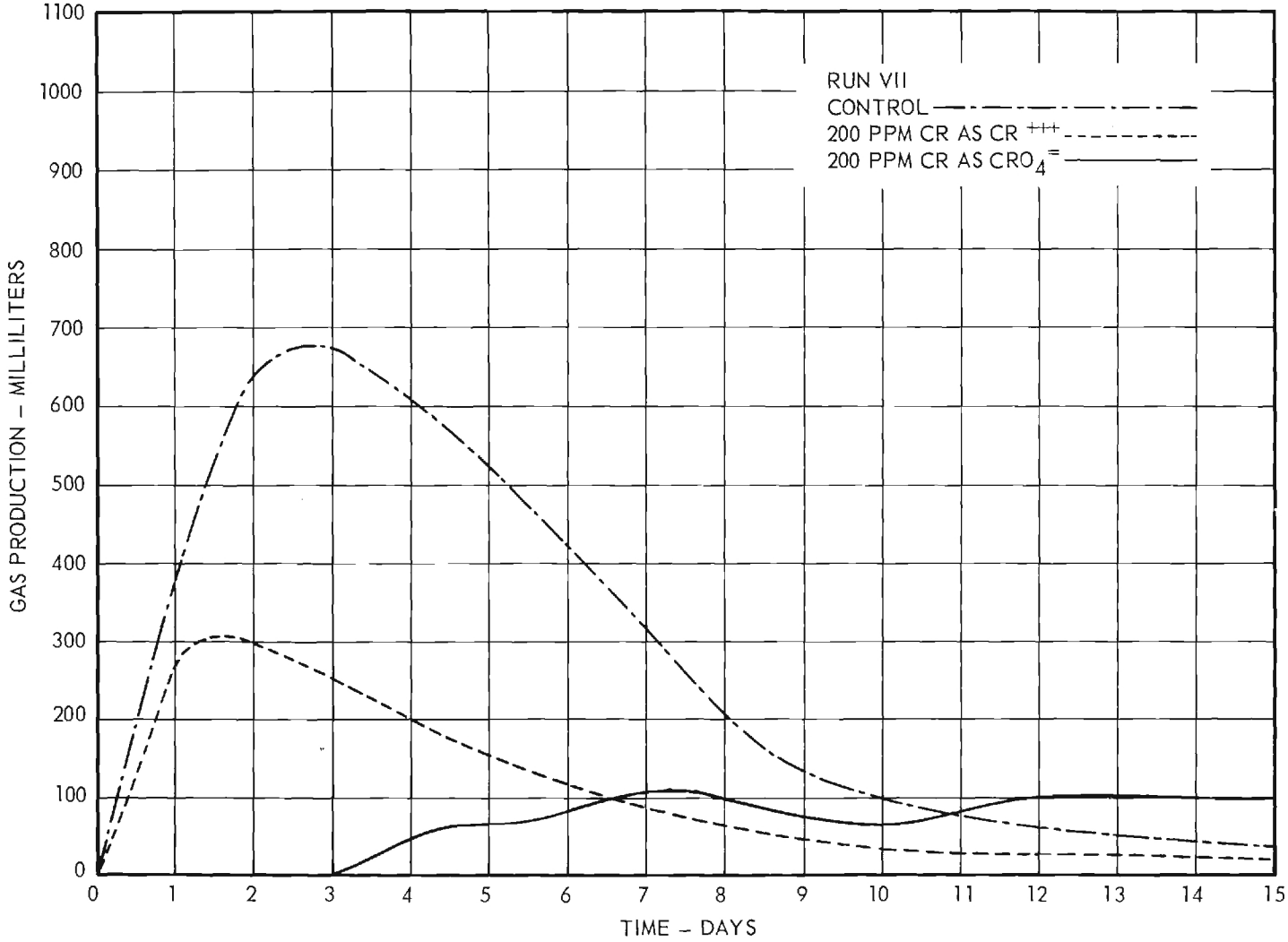

Figure 6. Comparison of Effects on Gas Production of 200 ppm Hexavalent Chromium and 200 ppm Trivalent Chromium in Run VII.

TABLE I

RELATION OF ORGANIC CONTENT OF SLUDGE
TO EFFECT OF CHROMATE ON SLUDGE DIGESTION

Run	Sample Size	Organic	Chromium Concentration Based on		Gas Production
			Total Sludge	Organic Content	
	(g)	(%)	(ppm)	(ppm)	(Per Cent of Control, 14 Days)
I	750	4.8	200	4,200	91
II	750	4.8	200	4,200	87
VII	1,510	2.4	100	4,200	85
V	1,010	3.6	200	5,600	57
VI	1,060	3.4	200	5,900	68
II	750	4.8	400	8,400	34
VII	1,510	2.4	200	8,400	21
IV	2,050	1.8	200	11,400	5
I	750	4.8	500	10,600	98†

†As indicated previously the data from Run I is completely out of line with most of the other information gained in this study.

which can be calculated from the oxygen added in the chromate ion. Thus, with 200 mg chromium (as chromate) the chromate contains 180 mg oxygen; where 200 mg chromium has been added to 36 g organic matter, the 180 mg oxygen could reduce the digestable organic matter by 2 per cent at the most. The gas production was decreased in two such situations by an average of 11 per cent. In another test, the gas production was decreased by 95 per cent where the organic matter could have been reduced by 6 per cent.

B. Differential Culture of Molds in the Presence of Bacteria

In those tests where chromate color persisted for a week or more and where, thereby, gasification was absent, an interesting observation was made. On the surface of the sludge mixtures, fungi or sporulating molds were observed to develop. Because of the differences noted in the response of "activated sludges" with different types of predominant organisms and because of other basic implications, it was considered necessary to study the fact that molds grew where there was a definite residual of chromate. Several Petri dishes were poured with a constant volume of sewage (1 ml) and nutrient agar with various amounts of sodium chromate. At 500 mg per liter sodium chromate, no visible bacteria colonies developed. Eventually slow growing molds appeared (within three or four days incubation). See Figure 7.

C. Studies with Activated Sludge

The studies with activated sludge were all performed on a floc maintained in the laboratory and fed with peptone, starch, and BOD mineral nutrients.† The same food was generally used during a toxicity study as was used to develop the floc so that the nutrient environment was normal to the organisms just as sewage and a sewage plant floc would have been.

Variations in respiration rates (as determined by carbon dioxide production) were used to measure chromium toxicity. The floc was settled, the supernatant decanted, and fresh BOD dilution water added to the floc. Various concentrations and types of food (the two chromium valences and different floc

† American Public Health Association, Standard Methods for the Examination of Water Sewage and Industrial Waste, New York City, 1955.

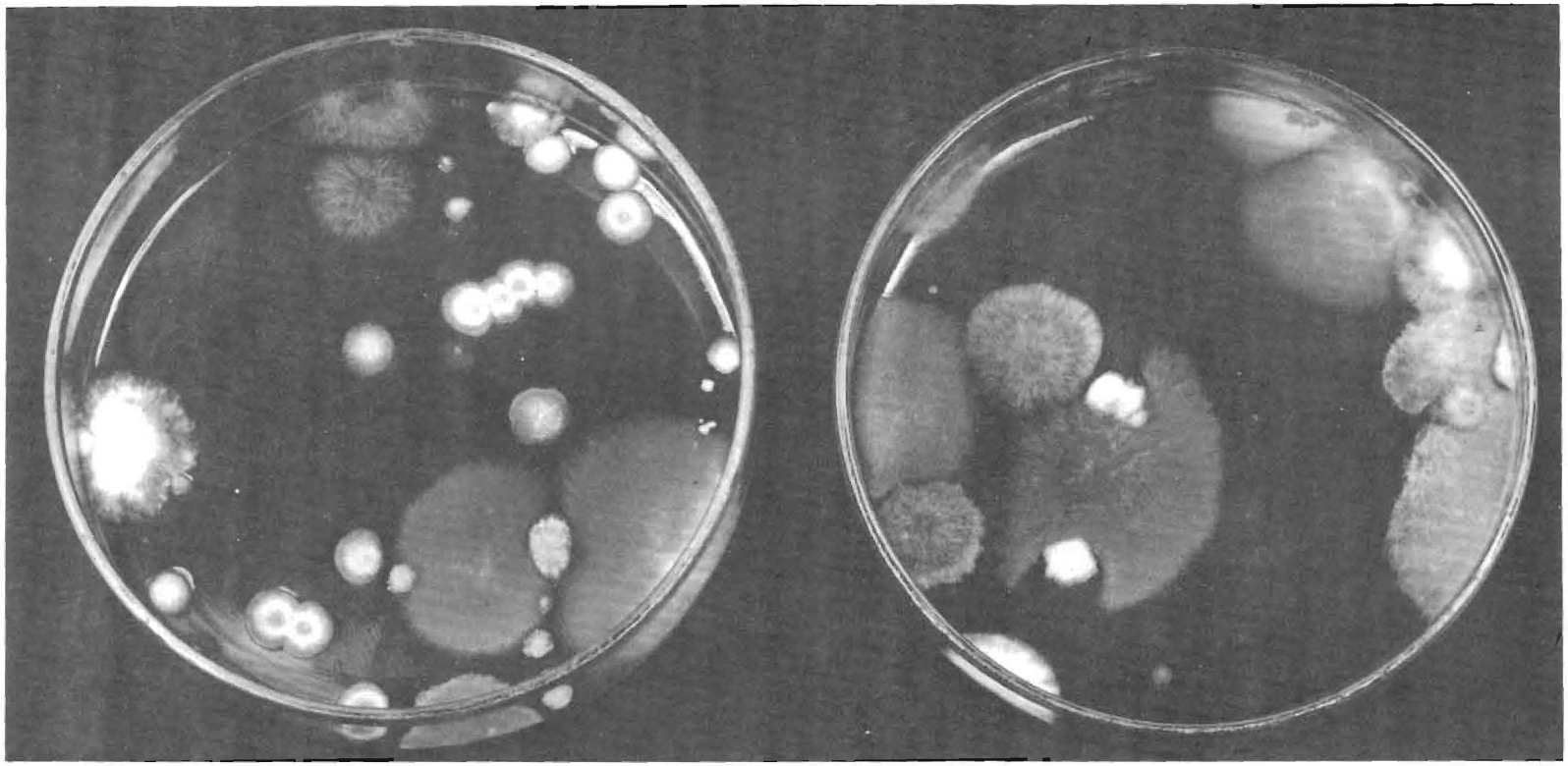

Figure 7. A Photograph of the Petri Dish Containing Dextrose Agar with 500 ppm Sodium Chromate and Innoculated with 1 ml of Sewage.

conditions) were used as factors to be studied. The aerators were placed in constant temperature baths for experiments with replicate flasks at different temperatures. In general, during the early studies, sufficient floc was taken from the large tank to yield approximately one gram per liter of suspended solids. After several months an experiment was run with sludge concentration as the only variable.

After considerable preliminary work the limitations of the equipment were established which were necessary for obtaining results to show equivalent increases in carbon dioxide production from relative increases in the food concentration at a given temperature and sludge concentration. It was found that carbon dioxide production failed to increase proportionately beyond a food concentration of 800 mg per liter with one gram sludge per liter. Thus, when it was necessary to increase the food to sludge ratio it was necessary to decrease the sludge concentration.

After many runs with different concentrations and types of food, many conflicting results were obtained. At times, the results indicated that with an increasing ratio of food to sludge, the higher food concentrations decreased the toxicity (see Figure 8). At other times there was a strong indication that the more food, the greater the toxicity of a given amount of chromate (see Figure 9). When nitrates were used as the only added source of nitrogen, there was very little consumption of the nitrates by the microorganisms (this was indicated by the carbon dioxide production similar to an aliquot of the sludge with sugar and no added nitrogen source). Under these conditions, with no reproduction, no toxicity was evident. When the sludge received only nitrates as a source of nitrogen for several days before the test, the carbon dioxide

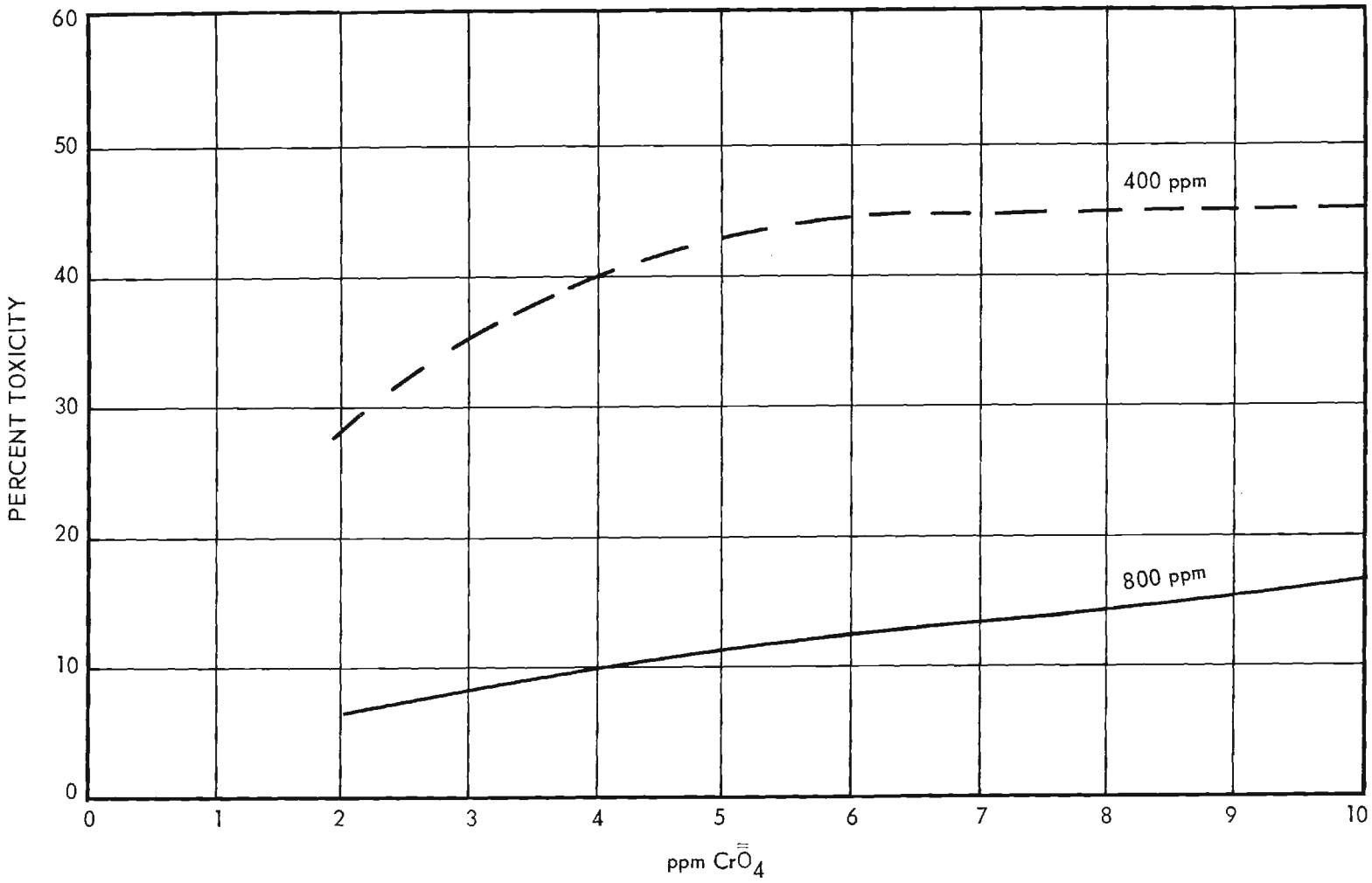

Figure 8. Effect of Food Concentration on Toxicity.

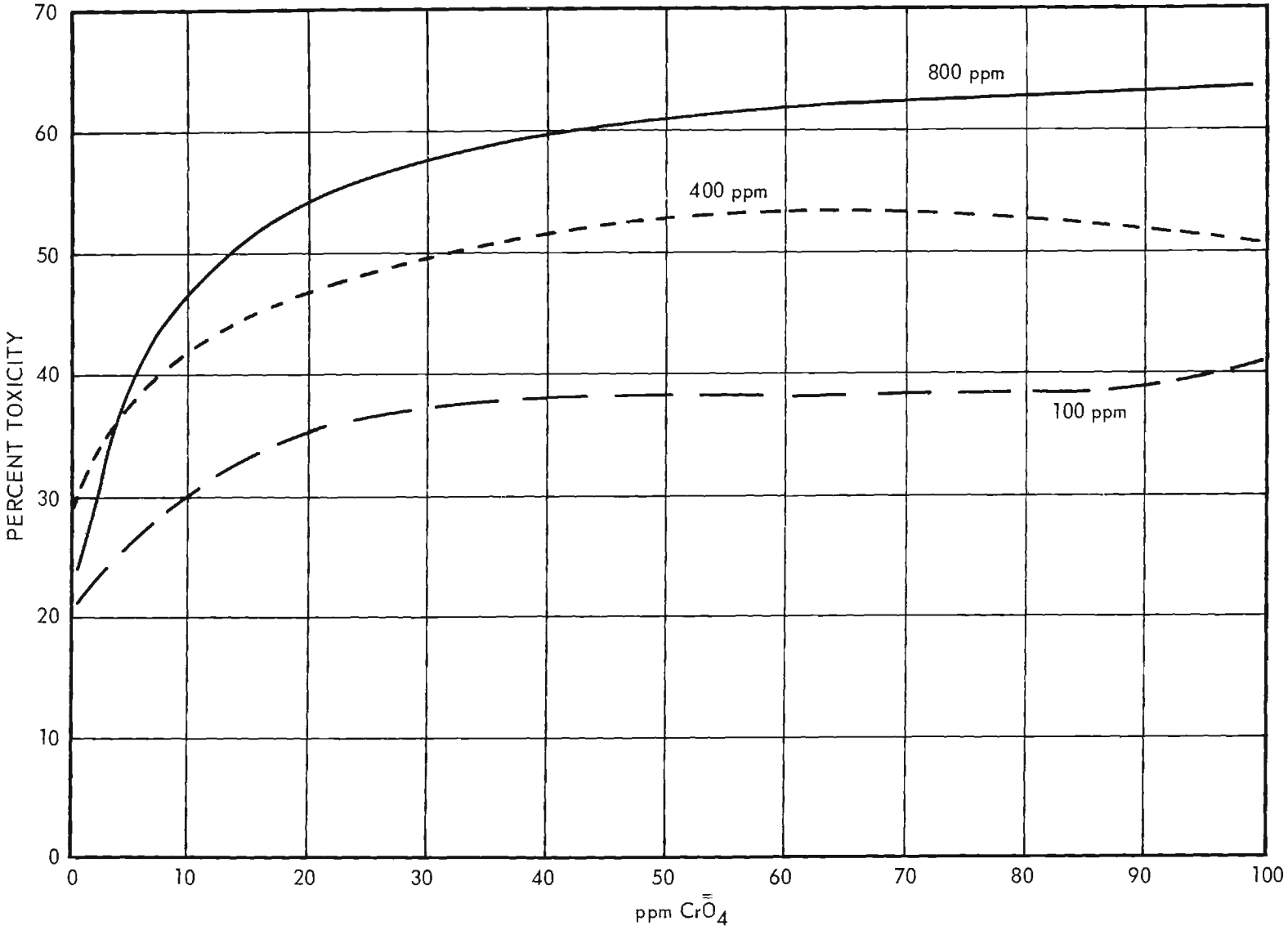

Figure 9. Effect of Food Concentration on Toxicity.

production in the control was much greater and there was obvious toxicity of the same order of magnitude as the toxicity obtained when ammonia was used as the source of nitrogen. These results indicated that chromate does not interfere with respiration except when conditions favor a high rate of respiration with reproduction.

When the aeration jars were held at three different temperatures, the toxicity increased with increasing temperature. This relationship is essentially the same as that demonstrated in the food concentration experiments. At 1° C there was very little toxicity, while at 37° C there was more than 50 per cent toxicity. By examining the individual hourly results of toxicity at different temperatures, it became evident that there was a maximum period of toxicity at high temperatures (37° C) and then a period when the mixture with chromate had a higher respiration than the controls. The relative lengths of the periods of toxicity and apparent stimulation are a function of food-sludge ratio. By comparing 10 hours at 10° C with 5 hours at 22° C with 3 hours at 37° C it became evident that there was much more toxicity at higher temperatures.

The problem of choosing a proper aeration period for comparing the control sludge respiration against the sample with chromate was brought out most forcibly by maintaining food and chromate constant and varying the sludge concentration. The results shown in Figures 10 and 11 indicate that a sludge with a high food concentration may respire at a high rate (milligrams carbon dioxide per milligrams sludge per hour) and not be affected by the chromate during the first hour. However, as the favorable conditions continue for a high rate of reproduction in the control (logarithimic phase), the chromate becomes progressively more toxic.

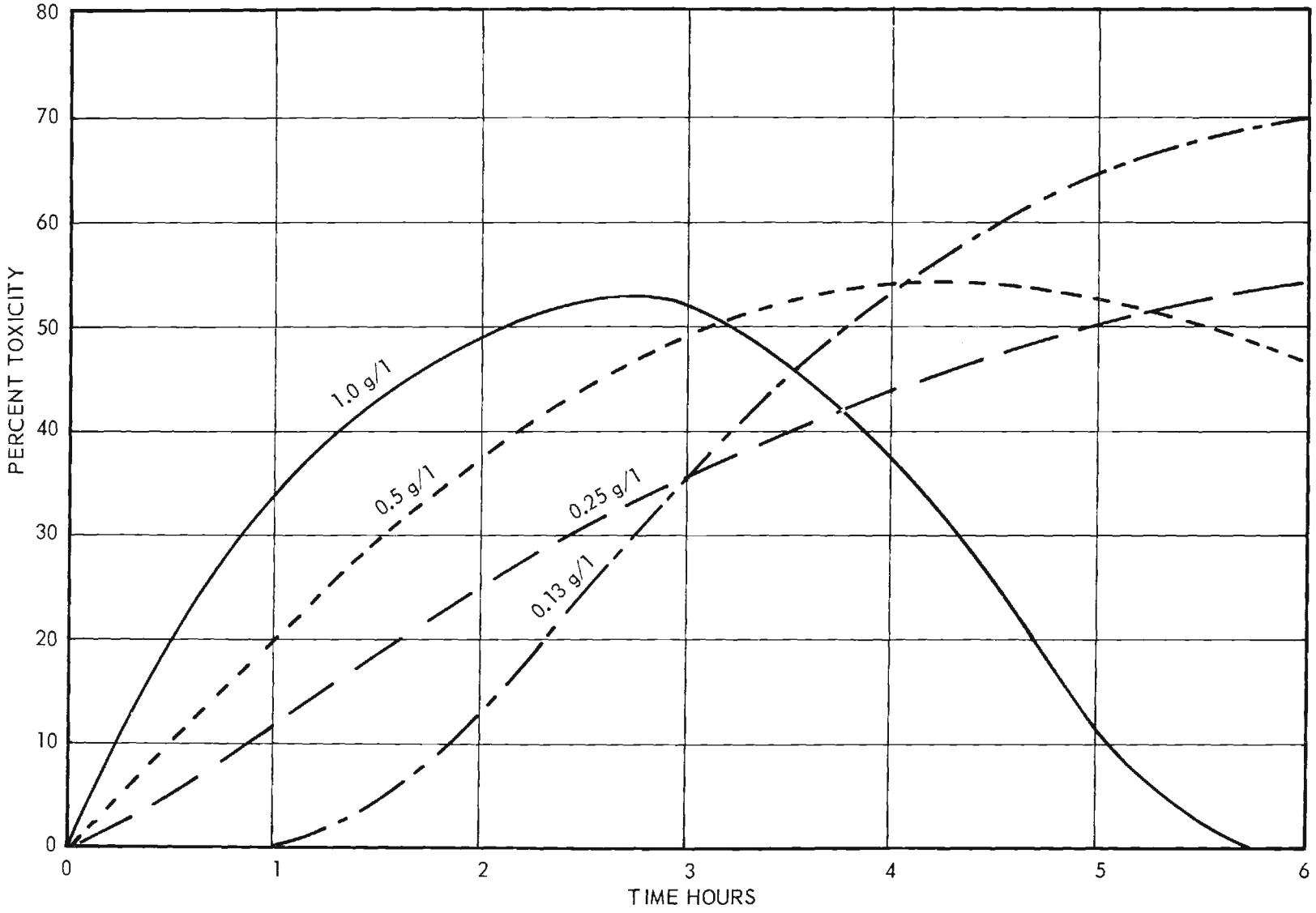

Figure 10. Relationship Between Toxicity and Time of Aeration of Various Sludge Concentrations.

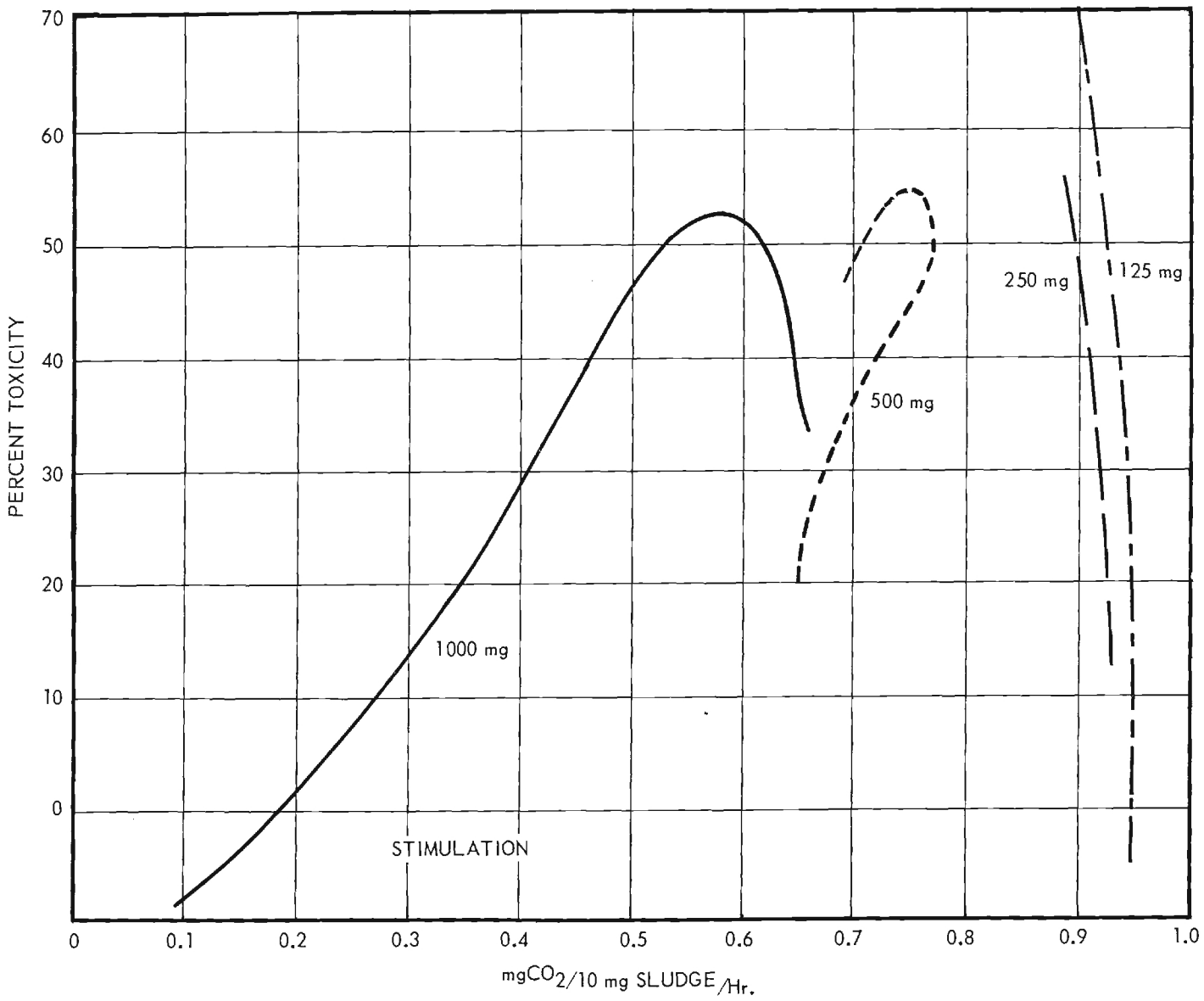

Figure 11. Relationship Between Respiration Rate and Per Cent Toxicity.

Because it has been frequently observed that organisms can develop a tolerance to a toxic element, it was considered desirable to try the effect of long term contact of activated sludge with chromate in low concentrations. Thus, sodium chromate was added to an aliquot of sludge to produce the low concentration of 10 mg per liter chromium at each batch feeding of the sludge. Within two or three feedings, the sludge became very bulky from an overgrowth of fungi. The basic cause for the predominance of the fungi over bacteria is explained by the pure culture studies given in the next section of this report.

The respiration studies comparing the treated and untreated sludges were difficult to interpret, but there was a clear cut evidence of a greater tolerance to the chromate by the treated sludge.

D. Pure Culture Studies

Because the sludge floc organisms cannot be counted accurately, it was decided to substitute a bacterial organism which could (1) respire readily at 24° C, (2) grow singly, and (3) be counted on solid media. Escherichia coli proved to be a desirable organism. With this organism the food concentration was maintained at a high level while the effect of several concentrations of chromate upon the total respiration over a 6-hour period was compared with the number of organisms able to reproduce after being in contact with the chromate for a 6-hour period. The results indicate that a chromate concentration of 10 mg per liter had very little effect upon the respiration, but that only one per cent of the organisms could reproduce after 6 hours contact with this low chromate ion concentration (see Figure 12). This has been repeated in several runs with the standard deviations represented by the vertical lines through the points in Figure 12. When these same tests were run with 10 mg of chromic

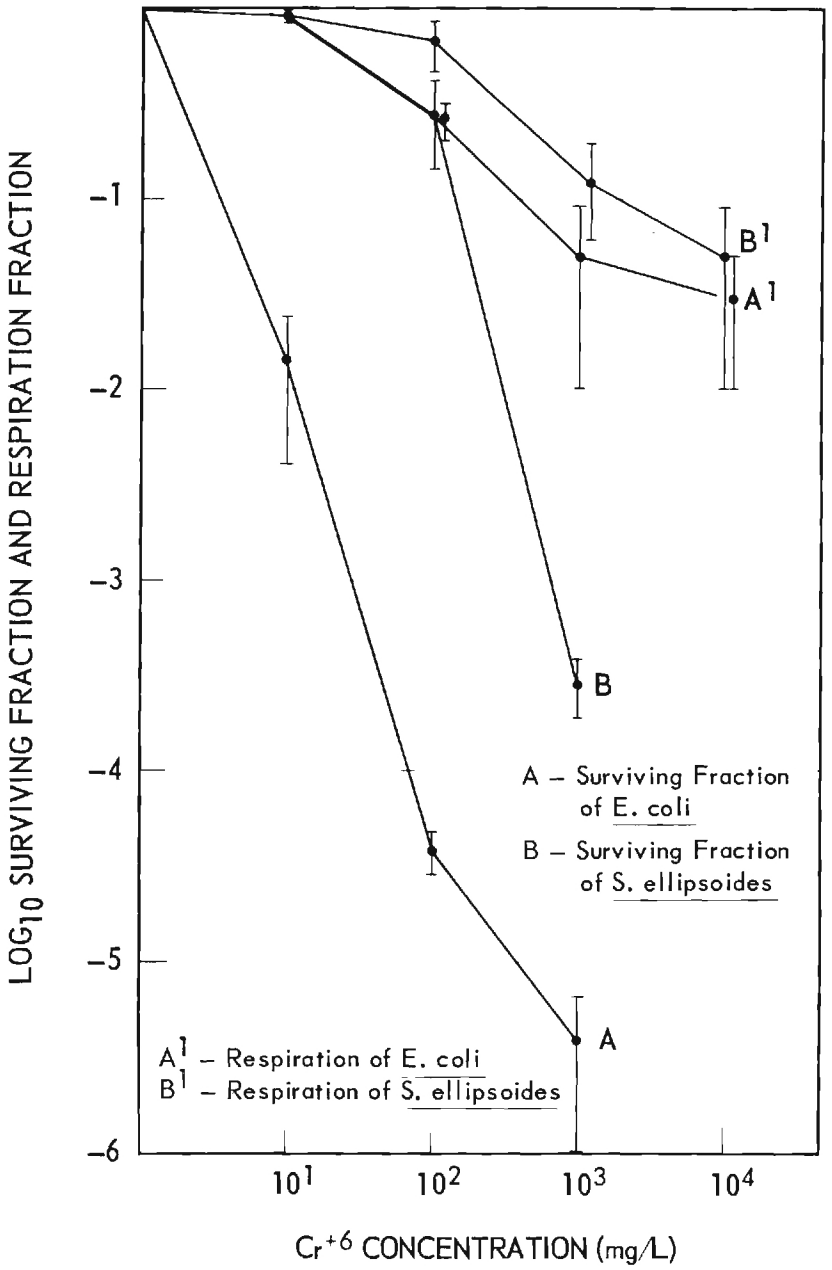

Figure 12. A Comparison of the Effect of Sodium Chromate at Various Concentrations Upon the Ability of Two Organisms, Escherichia coli and Sachramycetes ellipsoides to Respire for a 6-Hour Period and the Number of Organisms Which Will Grow Upon Nutrient Agar After the Respiration Study in Five Replicate Runs.

ions per liter there was no difference in numbers of viable organisms and very little effect upon the respiration of Escherichia coli and Azobacter agile. This would indicate that it is not the chromium which is toxic but the oxidation state of the chromate.

When the same criteria of desirable characteristics were applied to fungi, the Sachramycetes ellipsoides yeast was chosen. The chromate has much less effect upon the ability of the yeast to reproduce, as shown in line B, Figure 12. Other investigators studying the effect of mild oxidants have found similar results when comparing yeasts and bacteria.

Further studies, comparing respiration and surviving organisms, were repeated with the protozoan, Tetrahymena pyriformis, with results similar to those with the yeast. Apparently, the protozoans are less affected by mild oxidants than are bacteria. The generally accepted view that protozoans are more sensitive to chlorine than bacteria made this observation most surprising.

From the activated sludge and aerobic, pure culture studies, it may be concluded that (1) chromate chromium is more toxic than a similar concentration of chromic chromium, (2) the toxicity of chromates increases when the conditions for growth of the organisms become more favorable (that is, when there is an increasing temperature, food concentration and availability of nutrients, there is greater interference in respiration), (3) respiration mechanisms in bacteria are less sensitive to chromates than long term reproductive systems, and (4) the chromic ion shows no specific effect upon the reproductive ability of bacteria.

E. Transition from Aerobiosis to Anaerobiosis

This study was pursued for several months with very little success. When a sewage innoculum is used it is possible to obtain rather clear cut changes

from aerobiosis to anaerobiosis, as indicated by the single decolorization of methylene blue in the presence of nitrates. However, when an activated sludge innoculum is used the methylene blue color changes in intensity several times before being completely and finally decolorized. Some samples of sludge have caused an immediate decolorization of methylene blue to make it completely impossible to interpret a relationship of the chromate sample to the control. The cycling of color intensity was repeated in enough samples with enough replicates that it was felt certain that there was a true biological change and not an instrumental failure.

A few experiments with chromate were attempted but no results were obtained that could be used for a better understanding of the chromate toxicity.

VI. GENERAL DISCUSSION

The findings of generally greater toxicity of the chromate ion over the chromic ion for anaerobic conditions were expected from studies of the literature, but the findings of greater toxicity of chromate ions over chromic ions under aerobic conditions were not expected. The differences in reported results are apparently caused by different techniques of evaluating toxicity. In most earlier studies long term intervals of observations of continuous incubation were used for assaying the toxicity, but we have found that differences in rates of respiration may occur for only limited periods of time. Thus, the respiration rates during the third hour at low food to sludge ratio may show a high factor of toxicity while during the sixth hour there may be apparent stimulation. The stimulation is caused by the fact that the chromate has interferred enough with the respiration during the early hours of incubation, that there

is a higher food concentration in the mixture with chromate than in the mixture without chromate (control). The chromate does not generally interfere with low rates of respiration. This raises a problem in interpretation; what time period should be considered in the use of toxicity data in arriving at reasonable limits for quantities of industrial wastes to be added to sewage treatment plants? Should only hourly rates be considered, or a total respiration after 3 hours, or after 6 hours, or possibly 24 hours?

With different temperatures giving different rates of respiration in the control and different periods of high rate respiration where reproduction can take place, the choosing of a basis for evaluating toxicity becomes very significant. It is not certain that the correct choice of criteria has been made for the data of this report, but it is believed that the general conclusions are conservative.

VII. GENERAL CONCLUSIONS

It is believed that the most significant contribution of this report is the observation that the chromate ion interferes markedly in the reproductive metabolism of bacteria while interfering much less in the respiration, especially when the nutrient conditions are not favorable to reproduction.

Under anaerobic conditions, the persistence of any chromate ion greatly interferes with methane gas production. The ability of a sludge mixture to reduce the chromate largely determines the extent of suppression of gas production. A small amount of chromate will suppress a poor sludge digestion mixture while an active sludge digestion may tolerate 20 times the amount of chromate with virtually no effect.

Activated sludge can tolerate large amounts of chromium as chromate or chromic ion and continue to respire. However, when small amounts of chromate ion are added with each feeding there is a marked change from a bacterial floc to a fungus floc. At the very limited food concentrations of the dilution BOD test, the chromate shows less toxicity than the chromic ion, but at the high food to sludge ratios of this study the chromate ion is more toxic than the chromic ion.

Respectfully submitted:

Robert S. Ingols
Project Director

Robert H. Fetner
Assistant Project Director

Approved:

Wyatt C. Whitley, Chief
Chemical Sciences Division

J. E. Boyd, Director
Engineering Experiment Station